A spermatophore structured in the bursa copulatrix of the small white *Pieris rapae* (Lepidoptera, Pieridae) during copulation, and its sugar content

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Abstract. During copulation, the male small white, *Pieris rapae*, fills a single spermatophore in the bursa copulatrix of the female. The male fills first a white gel and then structures the spermatophore. The sperm was ejaculated last into the reproductive tract of the female, while sugars were observed throughout the copulation. Variation in size and sugar content of spermatophores observed in field-captured monogamous females indicated that sugars were consumed immediately after copulation, and that the spermatophore was gradually eroded. The role of the sugar content is also discussed.

Introduction

In butterflies, a spermatophore is passed during copulation. The size of the spermatophore decreases with successive matings (e.g. Pivnik & McNeil, 1987; Royer & McNeil, 1993). A number of studies have shown that males not only contribute sperm but actually make an investment by donating nutrients via the spermatophore (e.g. Thornhill, 1976; Boggs & Gilbert, 1979). Females absorb the nutrients and use them for somatic maintenance or egg production (Boggs & Watt, 1981; Marshall, 1985). The possibility that males may affect the rate of oviposition as well as contributing nutrients for egg production has implications concerning butterfly mating systems. In addition, oviposition is stimulated by the spermatophore (Watanabe, 1988).

Egg production requires protein-rich foods, the availability of which may critically constrain a female's lifetime reproductive success (e.g. Murphy *et al.*, 1983). Most investigations of spermatophore utilization have focused therefore on the use of amino acids by the female (e.g. Lai- Fook, 1984). Females incorporate amino acids from male ejaculates into their eggs and somatic tissue within 24hr after mating (e.g. Boggs & Gilbert, 1979; Boggs, 1981); receiving more of the non-sperm portion of the spermatophore has been shown to increase female fecundity (Oberhauser, 1989). On the other hand, Pivnik & McNeil (1988) demonstrated that males supplement the sodium requirements of the female via spermatophore transfer. Lai-Fook (1991) showed that labelled phosphorous from the male butterfly, *Calpodes ethlius*, was deposited in the reproductive tissues of the female. Zinc is also transferred to the female during mating (Engebretson & Mason, 1980). However, there are as yet no reports on sugar content in the spermatophore.

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In this study the sugar concentration of spermatophores is described, and their temporal changes during copulation in the small white *Pieris rapae.*

Studies of pierid butterflies have shown that spermatophores are a source of nutrition for the female during mating (e.g. Rutowski, 1984). In *Pieris* spp., oviposition increases with the number of spermatophores (Ando & Watanabe, 1992; Watanabe & Ando, 1993; Wiklund *et al.*, 1993). In *P. brassicae,* the secretion of the glandula supplies nutrients to the sperm stored in the spermatheca (Tschudi-Rein & Benz, 1990).

Here, we examine the transmission process of the spermatophore with specific attention to sugar content that may affect energy for sperm and/or for somatic maintenance of the female in *P. rapae.* We also evaluate the spermatophore decline with respect to the fate of the sugar in wild females.

Materials and Methods

P. rapae were reared in the laboratory on cabbage leaves, with fluctuating conditions of light and humidity, and temperatures around 23 °C. Late last instar larvae were caged and observed until pupation, similarly pupae were observed for emergence. All adults in this study were starved after emergence and were numbered on the forewing with a felt-tipped pen 24 hr after emergence. We allowed mating between the virgin females and the virgin males (one day old in both cases) kept together in a greenhouse. All began to copulate within 30 min in the mating greenhouse; the duration of copulation was recorded. Females used in these experiments had been kept in the laboratory environment from at least the 4th instar stage and were chosen without regard to size, because they varied little (ca. 27.5 mm in forewing length).

Spermatophore transmission in the female was observed by artificial interruption of copulation. We allowed pairs (one day old) to engage in the following kinds of copulation: 10, 20, 30, 40, 50, 60, 70 or 80 min interrupted copulations (total 121 pairs) and uninterrupted copulations (31 pairs). Both females and males were weighed just before release into the mating greenhouse, and again immediately after the copulation (±0.1 mg). Abdomens of both sexes were picked apart with a pair of tweezers, and dissected at various intervals during copulation. The volume and sugar content of the ejaculate were measured. When a spermatophore was structured in the bursa copulatrix, the volume was recorded by putting it into a glass tube with a known volume of distilled water under a dissecting scope. However, when there was no spermatophore structured, the volume of ejaculate donated by the male was regarded as a difference between the bursa copulatrix with ejaculate and the bursa copulatrix without ejaculate, both of which were recorded by putting them into a glass tube with a known volume of distilled water under a dissecting scope. Any sperm present were observed under a light microscope at 600×.

Sugar content of each ejaculate was analyzed using the Sugar Analyzer (YSI-MODEL 27). Single ejaculates were macerated in 75 µl of distilled water and homogenated. Each 25 µl of the suspension was examined for the sugar weight contained, using the Sugar Analyzer and then summed. This technique enables quantification of sucrose, with an accuracy of 0.25 µg/25 µl.

P. rapae females were collected in the field during the summer of 1993, in cabbage fields of Shirouma, Nagano Prefecture, in the cool temperate zone of Japan. They were sufficiently abundant to allow field collection. Females captured were classified

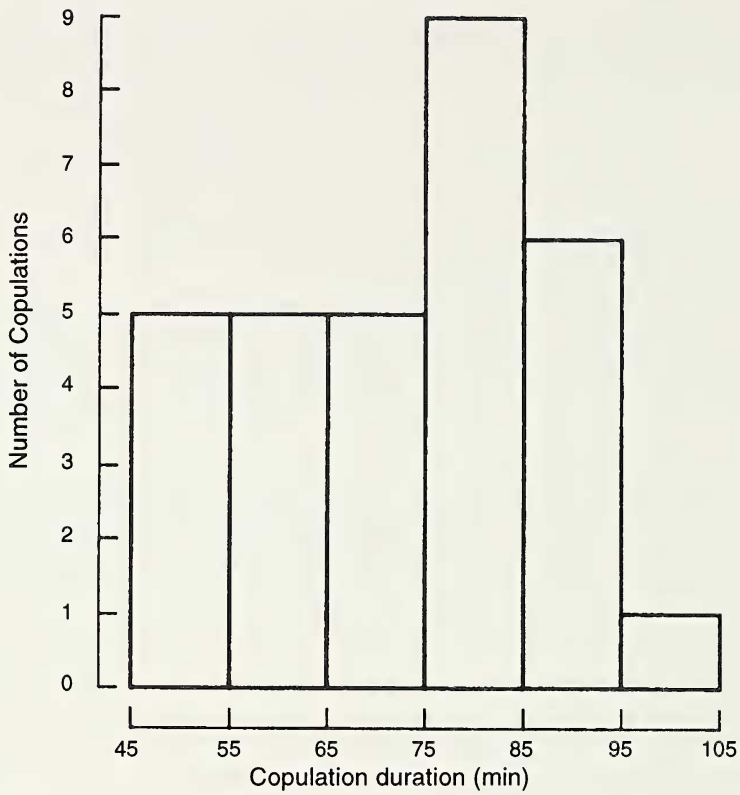

Figure 1. Frequency histograms of durations of successful copulations.

into 5 age groups (O-IV), on the basis of the degree of wing wear (Watanabe & Ando, 1993). After the abdomens were amputated, all spermatophores were removed by dissection. The volume and sugar content of the spermatophores were measured. Each sugar concentration was transformed by angular transmission.

Results

Laboratory experiments

Uninterrupted copulation durations were obtained for 31 pairs (Fig. 1). All were successful copulations, lasting 73.4 ± 14.7 min (SD). The spermatophore was produced directly in the bursa copulatrix and was filled with white secretion and sperm. The sperm sac was an elongated cone which occupied the bursal duct and had its opening at the end of the duct near the seminal duct. The volume of the spermatophore was 4.8 ± 0.29 mm^3 (n=31, SE). The weight loss by mated males was 8.9 ± 0.41 mg (n=24, SE), while the weight increase by the mated females was 5.8 ± 0.60 mg (n=21, SE). During courtship and copulation, males of *P. rapae* were active, as if in flight. In this study, most males fluttered their wings during copulation, and some tried to fly with their mate. However, all the females were inactive during the copulation. Jones *et al.* (1986) suggested that weight losses include loss in the mating trial. Therefore, mean wet mass of the spermatophore in this experiment was

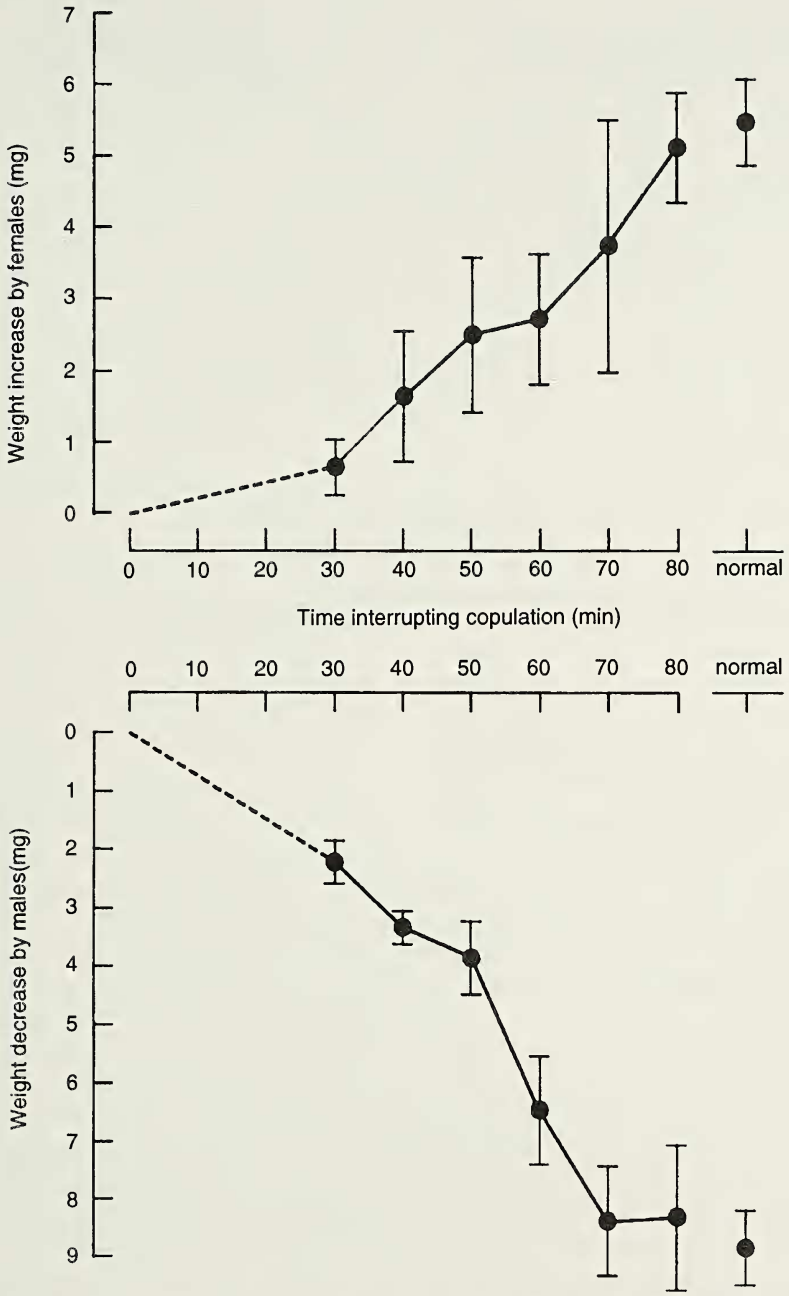

Figure 2. Weight increase by mated females and loss by the mated males during interrupted and uninterrupted (=normal) copulations (± SE).

about 5.8 mg. The mean body mass of the virgin males and females was 79.0 ± 2.63 mg (n=28, SE) and 81.6 ± 2.67 mg (n=23, SE), respectively. The mass of a spermatophore, plus appendix bursa contents, represents 7.3% and 7.1% of a body mass of males and females respectively.

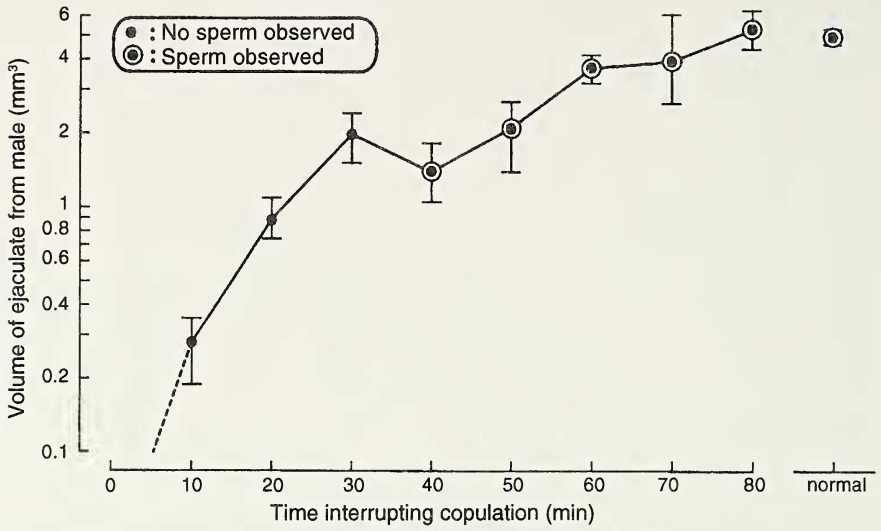

Figure 3. Volume of ejaculate transferred from male during interrupted and uninterrupted (=normal) copulations (± SE).

There was no detectable weight loss by mated males 20 min after the beginning of copulation (Fig. 2). The weight thereafter began to decrease. The loss was about 8 to 9 mg in 70 min or 80 min of interrupting copulation, each of which was not significantly different from that of normal copulations ($F=1.580$ for 70 min, $F=0.651$ for 80 min). The weight of mated females did not increase 20 min after the beginning of copulation, suggesting that little transfer from male to female occurred during the first 20 min of the copulation. The weight increase at 80 min of interrupted copulation was not significantly different from that of normal copulation ($F=1.157$).

During copulation, the male secretions from the ductus ejaculatorius and accessory glands were transferred serially to the bursa (under soft X-ray: unpublished). The ejaculate was a white gel mainly observed on the tip of the penis 10 min after the beginning of copulation. There was no spermatophore sac in the bursa copulatrix. No sperm were stored in the bursa copulatrix before 40 min (Fig. 3), indicating that males did not transfer sperm during this period. A spermatophore sac containing sperm appeared 40 min after the beginning of copulation. It seemed that the surface of the white gel solidified and became the spermatophore sac. The spermatophore increased in volume until 60 min after the beginning of copulation, which was not significantly different from that of normal copulation ($F=0.516$).

Sugars in the ejaculate appeared 10 min after the beginning of copulation (Fig. 4). The weight was relatively constant until 50 min after the beginning of copulation, and then it increased. There seemed no relation between sugar weight and the onset of sperm transfer.

As shown in Fig. 5, a relationship between the volume and the weight of ejaculate was observed. The latter was considered as the weight increase of females during the copulation. The sugar concentration (w/w) for each

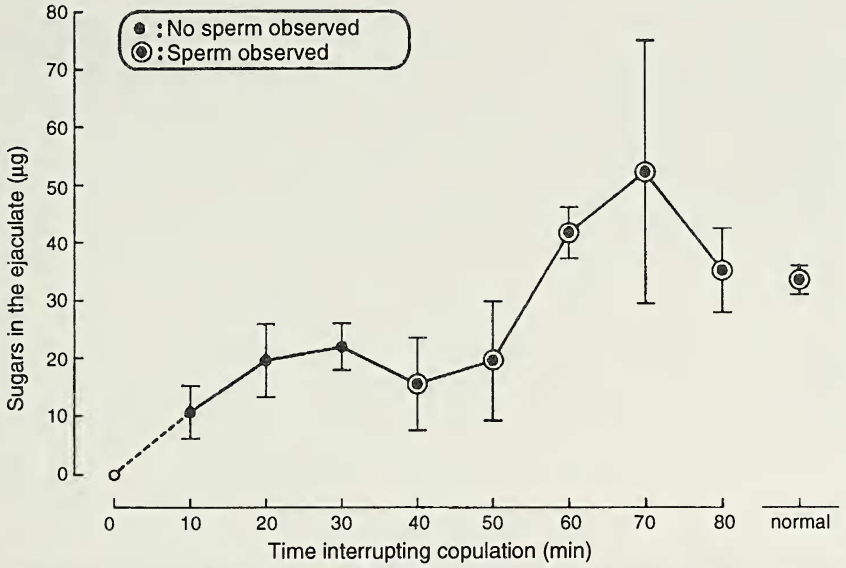

Figure 4. Sugars in the ejaculate transferred during interrupted and uninterrupted (=normal) copulations (± SE).

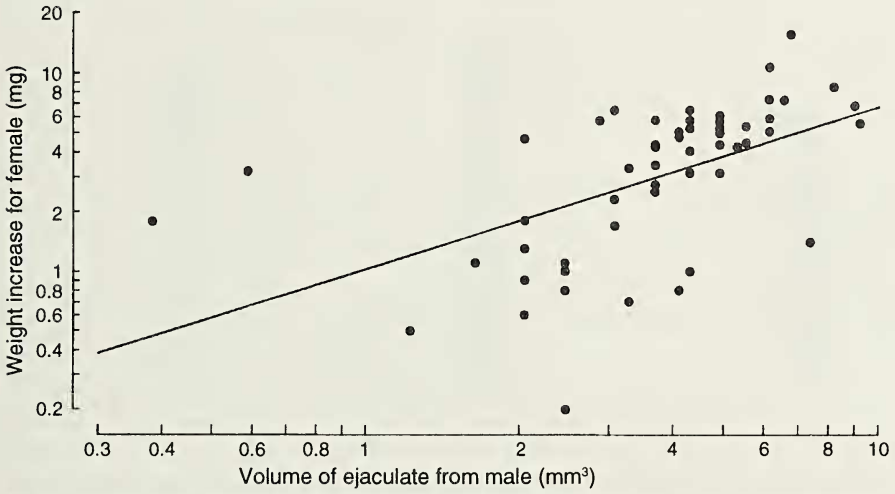

Figure 5. Results of volume of ejaculate transferred from the male during interrupted and uninterrupted copulations (V), shown as weight increase in the female (W) with least squares regression line (log W = 0.03 + 0.79 log V, r^2=0.29, P<0.001).

ejaculate was calculated using the regression in Fig. 5. It decreased with time after mating (Fig. 6).

Sugar content and characteristics of wild-caught females

Out of 43 females captured in the field, 11 were young (age O) and 4 were the old (age IV). Monogamous females were found in age O (n=9), I (n=4)

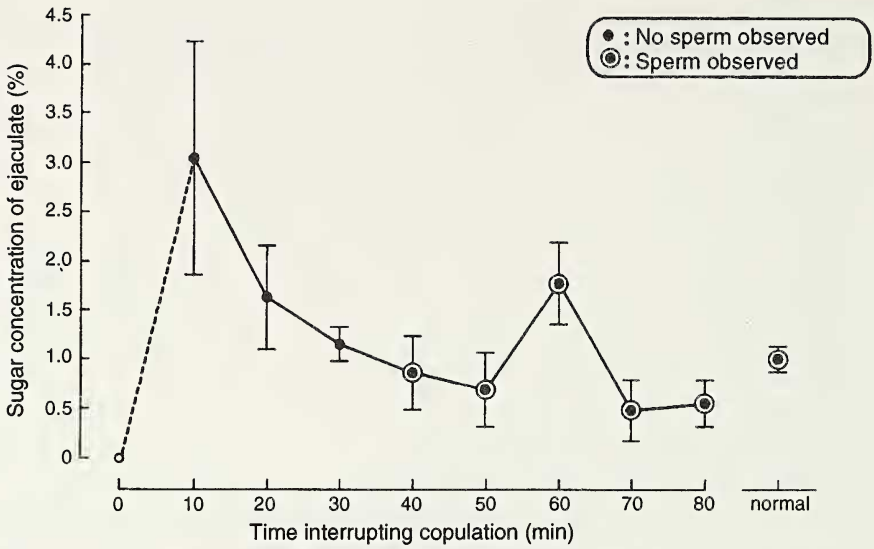

Figure 6. Sugar concentration of ejaculate transferred from the male during interrupted and uninterrupted (=normal) copulations (± SE).

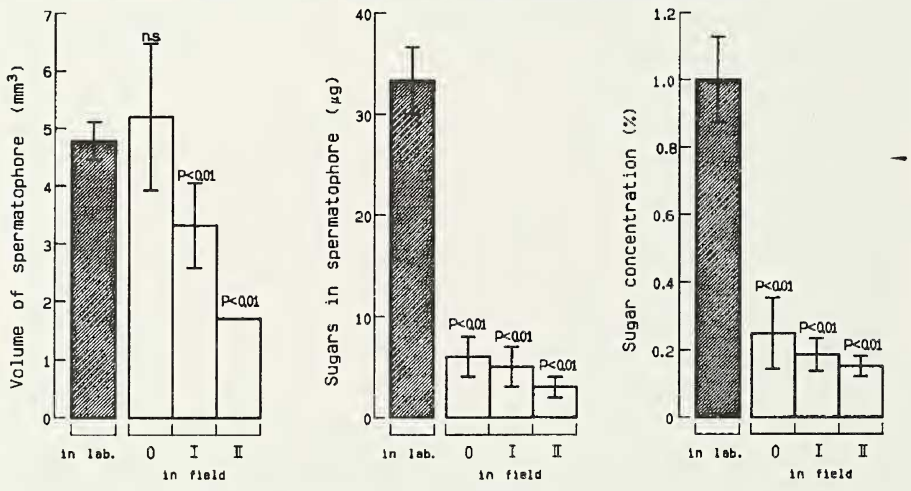

Figure 7. Spermatophore depletion as measured by mean volume, mean sugar content and mean sugar concentration (± SE). Spermatophores in lab. were collected from females of uninterrupted (=normal) copulations. O, I and II in field refer to monogamous female ages, in which a spermatophore was collected.

and II (n=3), and the others were polyandrous. As shown in Fig. 7, spermatophores in the females of age O were the largest among three age groups of monogamous females. The volume was not significantly different from that of the laboratory-reared females (F=1.054, d.f.=1, 30). However, the spermatophores in the female of age I and II were smaller than those in laboratory-reared females (F=6.203, d.f.=1, 25 for age I and F=8.579, d.f.=1,

24 for age II). This indicates that the females gradually absorbed the spermatophore.

Sugar in the spermatophore was less than 10 μg in the wild females, which was significantly different from that of those laboratory-reared ($F=18.731$ for age O, $F=14.050$ for age I and 4.597 for age II). Sugar may thus be consumed upon termination of copulation. Sugar concentration in the spermatophore in the wild females of each age was also lower than that in the laboratory-reared ones.

Discussion

During a copulation, a male *P. rapae* fills the appendix bursa with a white substance containing sugars, and constructs a spermatophore in the bursa copulatrix. This deposition represents approximately 7% of the male's body mass. Marshall (1985) reported that 6 to 7% of the male's body mass was ejaculated in *Colias philodice* or *C. eurytheme.*

Rutowski & Gilchrist (1986) suggested that duration of copulation is relatively long as a result of the mechanical problems of filling the bursa copulatrix. However, in the silkworm, *Bombyx mori,* ejaculation of seminal fluid into the spermatophore terminated 20 min after the beginning of copulation (Osanai *et al.*, 1986). In *P. rapae,* a little ejaculation was already observed 10 min after the beginning of copulation. In a copulation of normal duration, substances are passed to the female continuously throughout the copulation period. In addition, no male held onto the female before initiating the transfer of substances. This suggests that the male was not engaged in copulatory mate guarding.

The pattern of secretion production observed in *P. rapae* was similar to that of other Lepidoptera. For example, sperm were the last materials transferred to the female reproductive tract in *C. eurytheme* (Rutowski & Gilchrist, 1986). In the present study, when the copulation was prematurely terminated (until 30 min after the beginning of copulation), the male had passed some nutritious material, but none of his sperm.

Sperm are generally transferred to the spermatheca during the hours immediately following the copulation. Tschudi-Rein & Benz (1990) reported that sperm of *P. brassicae* was transported to the spermatheca 5.5 to 8 h after copulation. Sperm of *P. rapae* may stay in the spermatophore for several hours after copulation, during which time they must remain inactive. The spermatophore is a site of sperm maturation (e.g. Osanai *et al.*, 1987). Accessory gland products in spermatophores have been shown to function in sperm activation (e.g. Leopold, 1976). Sugar content in the spermatophore might thus contribute to sperm survival during this period.

Although it has been assumed that spermatophores are proteinaceous (e.g. Thornhill, 1976; Friedel & Gillott, 1977), Marshall (1980) has discovered that in *C. philodice* these accessory gland secretions are a complex of proteins, hydrocarbons, triglycerides, diglycerides, sterols and phospholipids. It has been shown that males may supplement a variety of nutrient requirements of a female via spermatophore transfer (e.g. Engebretson &

Mason, 1980; Alder & Pearson, 1982; Marshall, 1985; Pivnik & McNeil, 1988). Since these nutrients are absorbed by the female, a number of studies were restricted to the evidence indicating paternal investment (e.g. Boggs & Gilbert, 1979; Boggs, 1981; Boggs & Watt, 1981) and the male's genetic return (e.g. Rutowski *et al.*, 1987). Wiklund *et al.* (1993) showed that polyandrous females of *P. napi* had higher lifetime fecundity than monandrous females, laying on average 1.61 as many eggs.

Ejaculate donated by male includes protein and lipid (e.g. Oberhauser, 1992), most of which are derived from leaves fed during the larval period. Such substances may also be transferred from pollen (e.g. Gilbert, 1972) or nectar (e.g. Baker & Baker, 1973). In this study since males and females were starved, all the substances produced by males originated in immature stages. Sugars in the ejaculate were produced by the male himself.

In this study, significant quantities of sugar were observed in ejaculates. Such sugar must dissolve in water, because a spermatophore is more than 80% water (Boggs, 1981). Sugar levels decreased after copulation in wild females, while the spermatophore size did not change in the females of age O. Therefore, there seem to be two ways in which the sugar was consumed: Sperm could be supplied for the energy required for its own survival, or alternatively, the female could be supplied with the energy for activity in the bursa copulatrix. Takeuchi & Miyashita (1975) reported that the bursa copulatrix bended or elongated the spermatophore in order to dissolve it after copulation. Mass and nitrogen content of the spermatophore decreased at constant rates until little material remained (e.g. Oberhauser, 1992). However, sugar might not contribute to female somatic maintenance or egg production. It is obvious that many questions remain unanswered about sugar in the spermatophores and their functions as paternal investment.

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